



GAPDH is determined to be a useful control antibody for studying Sickle Cell Disease in livers

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Abstract

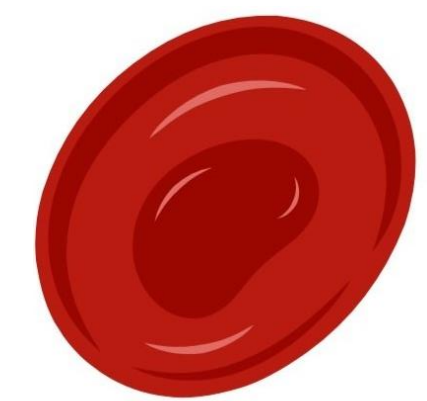
Using the Western blot procedure, a reliable control antibody was determined to be GAPDH when studying Sickle Cell Disease (SCD). Three primary antibodies were tested for their consistency in determining protein concentration in sickle cell mouse livers. As hypothesized, GAPDH accurately expressed protein concentration within the samples. Beta Actin was a less fit antibody because of its high presence within cytoskeletal filaments, which are lost during hemolysis of cells. KCTD12 was partially successful, likely due to its high presence in liver Kupffer cells. Determining GAPDH as a reliable primary antibody, the presence of desired proteins can be studied to monitor liver health in SCD patients.



Image 1. Rachel in the laboratory.

Introduction

- Hemolysis of sickled cells affects protein concentrations within the liver, impacting liver health.
- Protein concentrations are quantified through the Western blot procedure.
- The Western blot separates proteins found in samples by molecular weight.
- Antibodies are used to find desired proteins.
- A control antibody needs to be consistently present in the samples selected.



Normal red blood cell



Sickle red blood cell

Image 2.7 Depiction of healthy and sickle red blood cells.

Sickle Cell Disease

- Sickle Cell Disease (SCD) is a genetic mutation of the hemoglobin protein that distorts red blood cells into a sickle shape.¹
- SCD affects more than 100,000 people in the US and 8 million people worldwide.¹
- The impacts of SCD on the liver and treatment options are under-studied.²

Question Addressed

What antibody works best as a Western blot control when studying sickle cell liver samples?

Hypothesis

GAPDH will work best as a control antibody because of its high presence in many common tissues.

Results

- GAPDH had consistent expression.

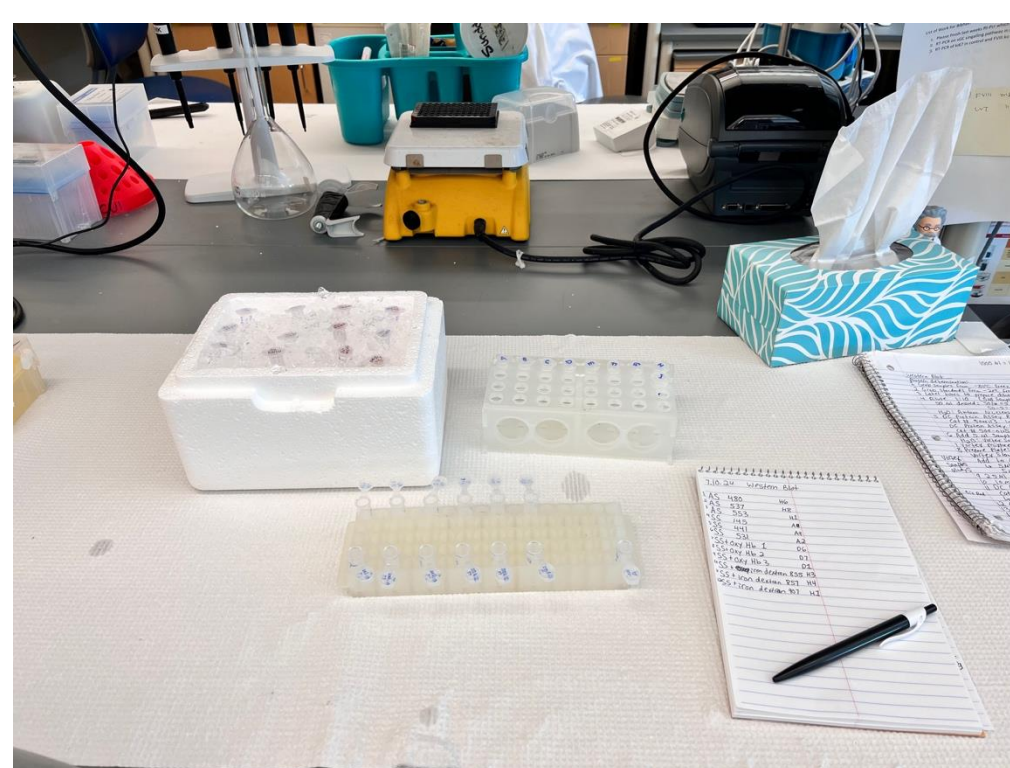
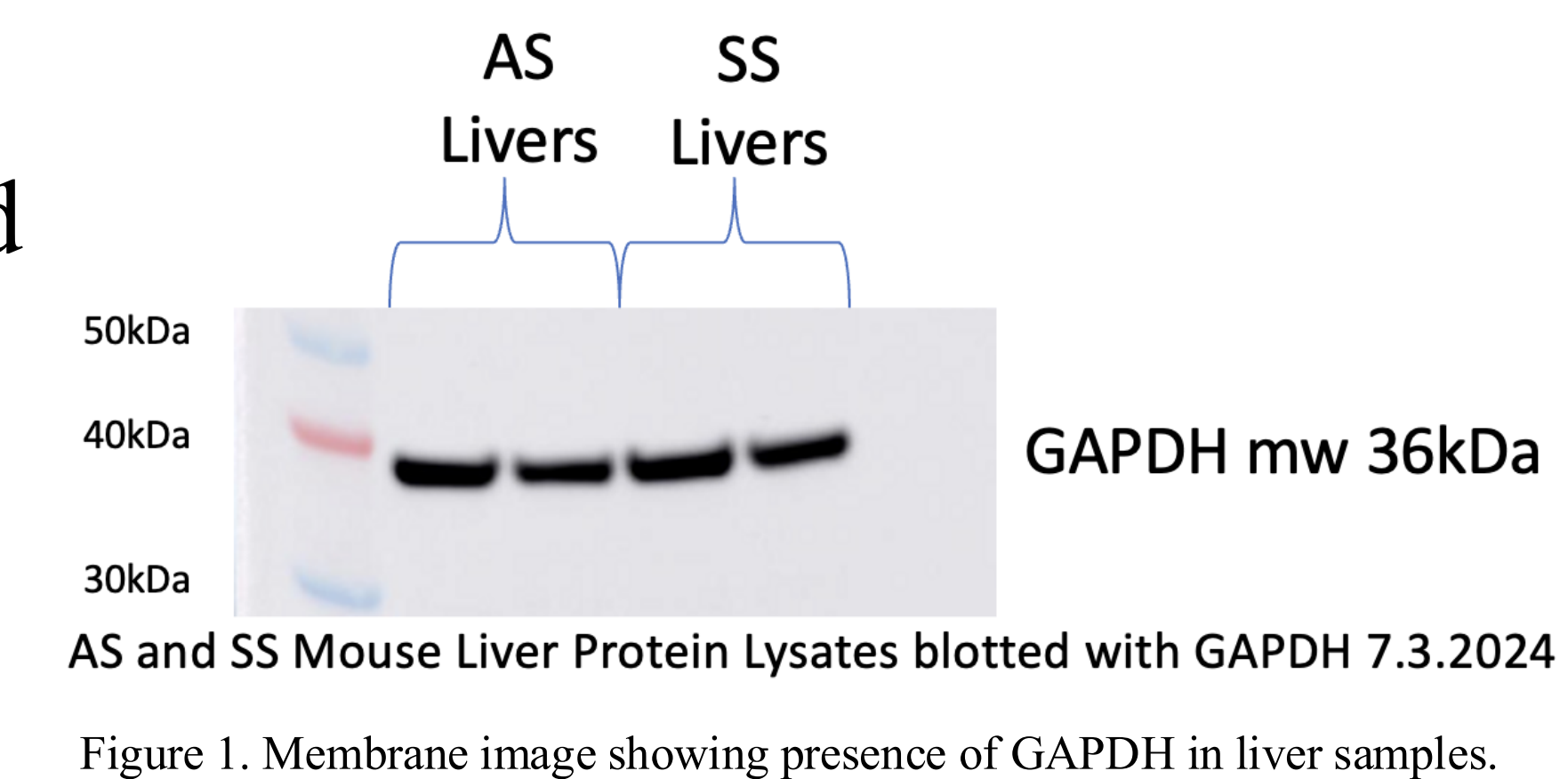


Image 3. Preparing the liver samples.

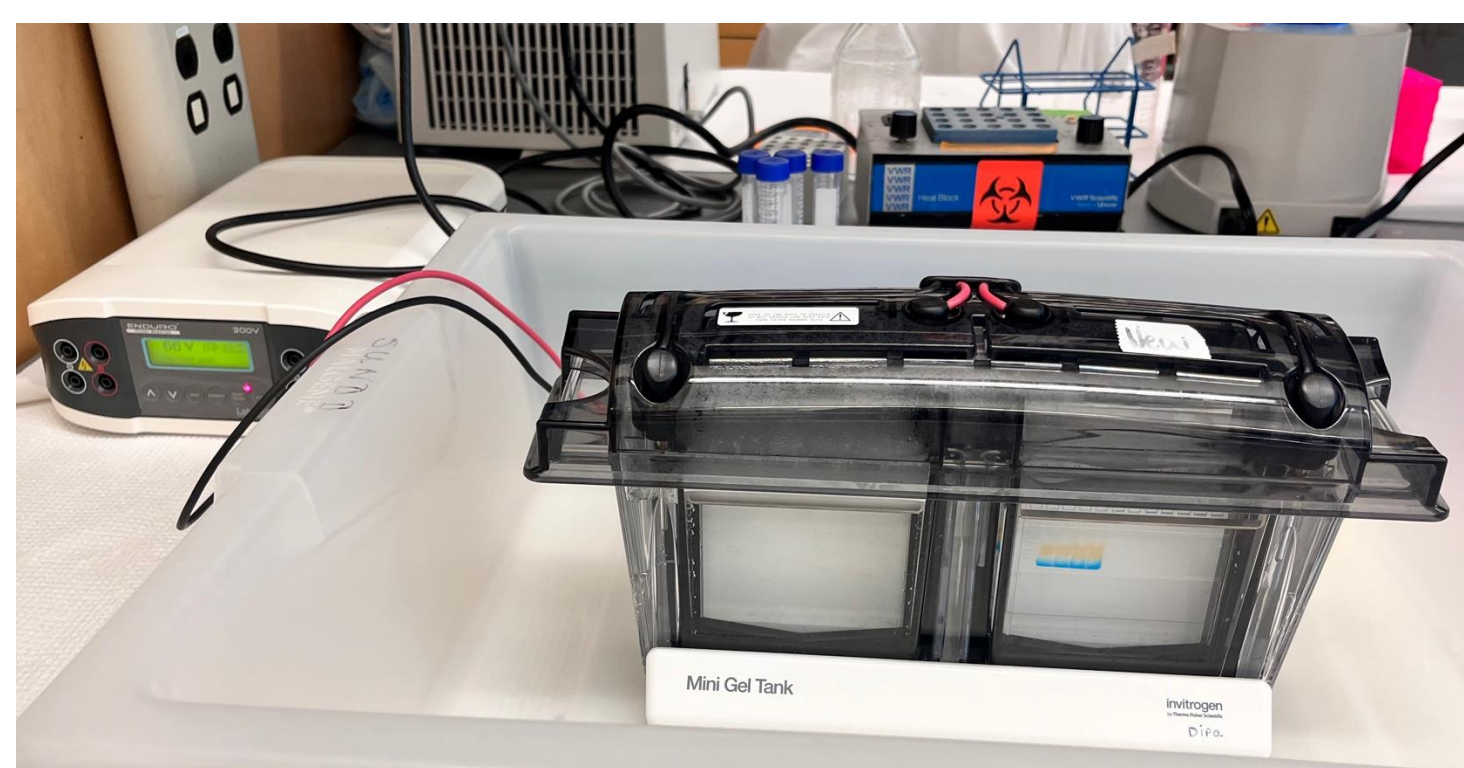
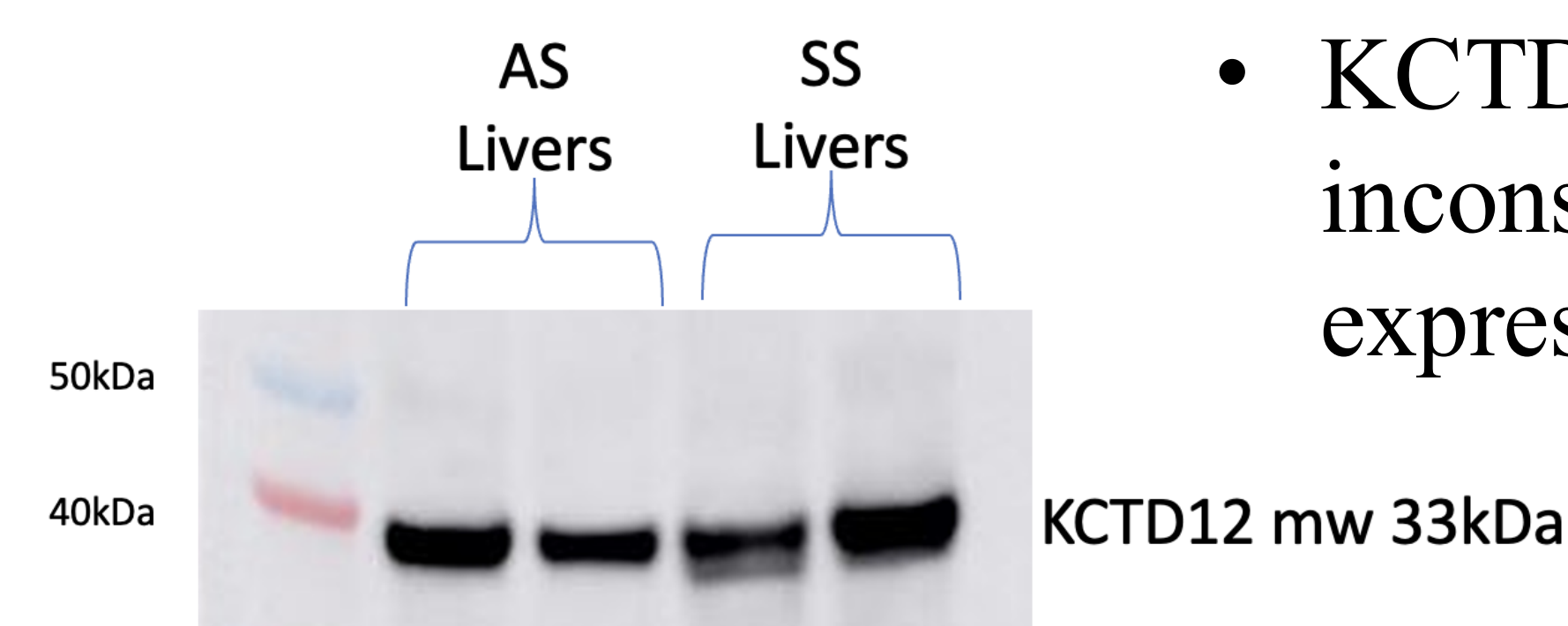
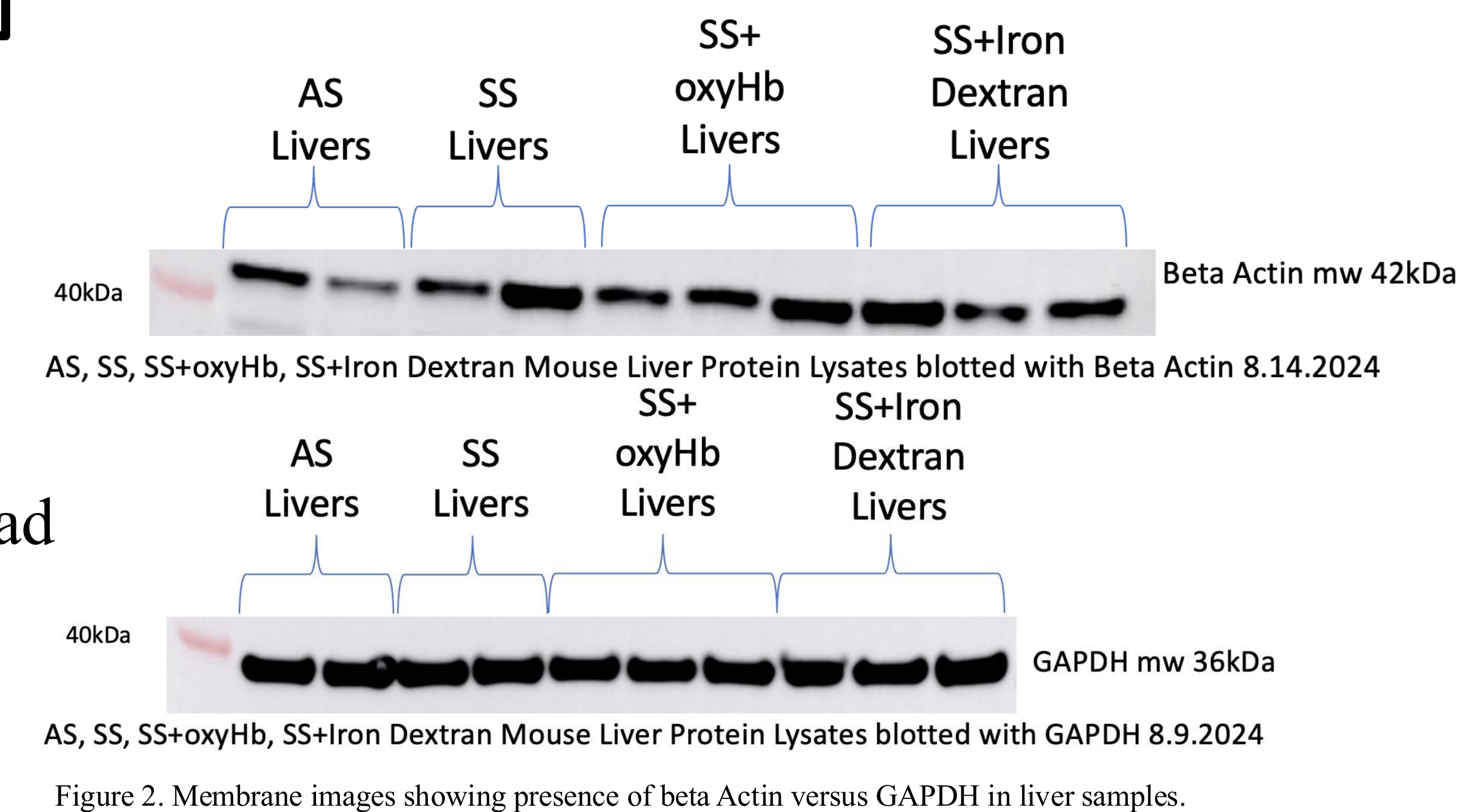


Image 4. Invitrogen Mini Gel Tank running a Western blot gel containing sickle cell mouse liver samples.



- KCTD12 had inconsistent expression.

- Beta Actin had inconsistent expression compared to GAPDH.



Methods

- Pre-harvested livers from lab mice with Sickle Cell Disease were utilized.
- Four treatments of livers were used.
- The Western blot procedure was conducted to separate and identify proteins.
- Three primary antibodies were tested to analyze their presence in sickle cell samples.
- A densitometric analysis was conducted on protein images to determine presence and concentration.

Discussion

- GAPDH is glyceraldehyde-3-phosphate dehydrogenase, and it is involved in many basic cellular functions.³
- Works well as a control antibody because of its high cellular concentration.
- Beta Actin is a major cytoskeleton filament protein.^{4,5}
- Is an inconsistent antibody because hemolysis in SCD breaks beta Actin.
- KCTD12 is potassium channel tetramerization domain containing 12, and it has no tissue specificity, but it is predicted to be localized in mitochondria.⁶
- Is an inconsistent antibody, although KCTD12 has unexpected expression in liver samples because it has high concentrations in Kupffer cells.

¹ National Heart, Lung, and Blood Institute. 2024. "Sickle Cell Disease - What Is Sickle Cell Disease?" www.nhlbi.nih.gov. NIH. September 30, 2024. <https://www.nhlbi.nih.gov/health/sickle-cell-disease>. ² Theocharidou, Eleni, and Abid R Suddle. "The Liver in Sickle Cell Disease." Clinics in Liver Disease 23, no. 2 (2019): 177-89. doi:10.1016/j.cld.2018.12.002. ³ "GAPDH Protein Expression Summary - the Human Protein Atlas." 2014. <https://www.proteinatlas.org/ENSG00000111640-GAPDH>. ⁴ "ACTB Protein Expression Summary - the Human Protein Atlas." n.d. <https://www.proteinatlas.org/ENSG00000075624-ACTB>. ⁵ Davis, S. 2022. "Beta Actin - What's so Special about It?" St John's Laboratory Ltd. February 9, 2022. <https://stjohnslabs.com/beta-actin-whats-so-special-about-it/>. ⁶ "KCTD12 Protein Expression Summary - the Human Protein Atlas." 2014. <https://www.proteinatlas.org/ENSG00000178695-KCTD12>. ⁷ Sickle. 2018. "THERAVIA." THERAVIA. 2018. <https://www.theravia.com/sickle-cell-disease>.